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Feeding modes of larvae of *Nematostella vectensis* (Cnidaria: Anthozoa)

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Abstract-

We assessed the ability of larvae of the starlet sea anemone, *Nematostella vectensis,* to assimilate dissolved organic material (DOM) and ingest artificial and natural particles from seawater. Planulae were exposed to the proteins ferritin and labeled bovine serum albumin (FITC-BSA) and the polysaccharides iron dextran and labeled dextran (FITC-dextran) at solute concentrations between 0.25-1.0 mg/mL for 1-5 hours at 22°C. Other larvae were incubated with polystyrene beads (0.5 µm, 10^6 beads/mL and 4.5 µm and 6 µm, 10^3 beads/mL) or with algal cells (*Dunaliella tertiolecta*, 5×10^3 cells/mL) for 2.5-5 h. The label from all provided macromolecules was detected only within the gastrovascular cavity. In intact and sectioned (1µm) larvae assimilation of ferritin was detected within cells of the pharynx and the endoderm. Assimilation of BSA-FITC was inferred from the presence of a diffuse fluorescence visible only in endodermal cells. The label from iron dextran and FITC-dextran was not detected within cells. Control larvae not exposed to provided macromolecules showed no detectable label. We found no particles in the gastrovascular cavity of larvae. These data indicate that particulate foods do not contribute to the energetics of larval development of *N. vectensis.* In contrast, planulae assimilated some forms of DOM (proteins) but not others (polysaccharides), suggesting that specific DOM could contribute to the energetics of larval development.

Introduction-

The feeding biology of planula larvae of anthozoan cnidarians is uncertain. The potential for larval feeding is suggested by the presence of an oral opening connecting the gastrovascular cavity (GVC) to the surrounding seawater, a morphological organization found in adult anthozoans (Fautin and Mariscal 1991). Digestion and absorption of particulate and dissolved organic matter (DOM) has been described for adult anthozoans (Schlichter 1975, Van Praët 1990), but consensus is absent if or how similar materials are collected by their larvae.

Earlier reports indicated that anthozoan larvae can ingest and digest particulate foods (Riggs 1989, Siebert 1974). Siebert (1974) observed the capture of 1-µm plastic spheres onto a mucus thread trailing the planulae of *Anthopleura elegantissima* and *A. xanthogrammica*, that was pulled into the GVC. Phagocytosis of zooxanthellae by the endoderm of larvae of symbiont-bearing anthozoans further supports the potential for particulate feeding by larvae of anthozoans (Schwartz et al. 2002, Weis et al. 2001).

We evaluated ingestion of particulate and macromolecular forms of DOM by larvae of sea anemone *Nematostella vectensis* and found that dissolved proteins were ingested and assimilated, polysaccharides in solution were ingested but not assimilated, and no forms of particulate matter were ingested.

Materials and Methods:

Culturing and Spawning:

Adult *N. vectensis,* provided by Adam Reitzel (W.H.O.I.), were maintained in filtered (0.2 µm pore size), artificial sea water (12% FSW) at 18 °C and provided daily with newly hatched *Artemia salina* nauplii. Spawning was induced by transferring adults to fresh FSW and warming to 24 °C.

Assimilation of Dissolved Organic Matter:

All experiments were performed using free swimming planulae (2 days after embryo collection). Individuals were exposed to two proteins (ferritin and FITC-bovine serum albumin (BSA)) and two polysaccharides (iron dextran and FITC-dextran) in seawater (0.25 mg/mL – 1.0 mg/mL) for incubations of 1-5 hours.

At the end of each incubation, larvae were washed in FSW, relaxed in $MgCl_2$, and fixed with 4% paraformaldehyde in HCO_3 buffered saline. To visualize iron from ferritin and iron dextran, specimens were incubated in a 3:2 mixture of 1% HCl and 2% potassium ferrocyanide for 1 hour. Experimental and control larvae were serially dehydrated with ethyl alcohol and were used for whole mount preparations or embedded in Embed 812 (EMS) for sectioning.

To detect the presence of the fluorescence in experimental and control larvae, specimens were washed in FSW, relaxed in $MgCl_2$ and examined using a Nikon E600 compound microscope (FITC, Ex: 330-380 nm; Em: > 420 nm).

Ingestion of Particulate Material:

Larvae were incubated with 0.5 µm fluorescently-labeled (10^6 beads/mL), 4.5 µm (10^3 beads/mL) or 6 µm (5×10^3 beads/mL) polystyrene beads (Polysciences) for 0.5-5 hours. Prior to each experiment with larvae, the beads were incubated for 2 h in 2.5 mg/mL BSA and then washed and resuspended in FSW. Planulae were also incubated with *Dunaliella tertiolecta* (UTEX strain LB 999, 5×10^3 cells/mL) for 0.25, 0.74, 1, 1.25, and 7 hours.

The fluorescence of 0.5 µm polystyrene beads was detected using a Nikon E600 compound microscope (FITC, Ex: 330-380 nm; Em: > 420 nm). *D. tertiolecta* were similarly visualized using chlorophyll autofluorescence.

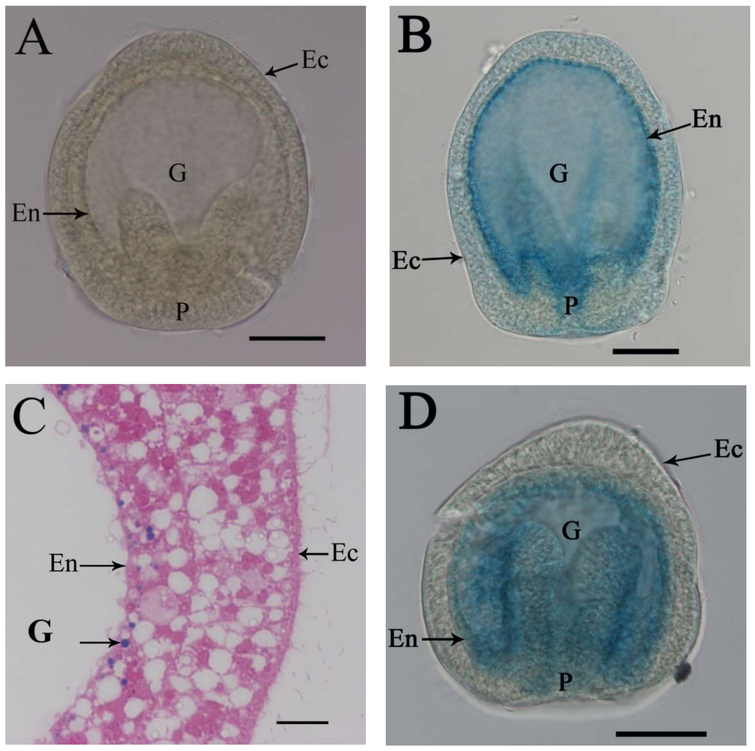

Fig. 1A: Light micrograph of a control planula. **B**: Light micrograph of a planula exposed to ferritin for 5 h. **C:** Light micrograph of a section of a planula exposed to ferritin for 5 h. **D:** light micrograph of a planula exposed to iron dextran for 5 h. G= gastrovascular cavity, P= pharynx, En= endoderm, Ec= ectoderm. Scale bars: Figs. 1A, 1B, 1D = 50 µm, Fig. 1C = 16 µm.

Results-

Protein Assimilation:

No label was found in control larvae, unexposed to ferritin (Fig. 1A). The blue reaction product from ferritin was concentrated as discrete dots in the ectodermal pharynx and endodermal lining of the GVC (Fig 1B); the abundance of the label was consistently greater in the pharynx. In sectioned larvae, the blue spots were present only within pharyngeal and endodermal cells (Fig. 1C). The blue reaction product was absent in the outer (nonpharyngeal) ectodermal epithelium. Individuals exposed to FITC-BSA showed a diffuse fluorescence within the GVC as well as fluorescent vesicles in the epithelia of the pharynx and the GVC (Fig. 2A).

Polysaccharide Assimilation:

Individuals (ca. 75%) exposed to iron dextran typically showed a diffuse blue color of the reaction product within the GVC. However, no label was detected within cells (Fig. 1D). Larvae incubated with FITC-dextran showed a bright, diffuse fluorescence within in the GVC for all time points, but there was no evidence of vesicles containing FITC-dextran within the cells of the pharynx or that line the GVC (Fig. 2B).

Ingestion of Particulate Material:

When incubated with protein-coated polystyrene beads and with algae *Dunaliella tertiolecta*, no particles were seen within the GVC of the planulae.

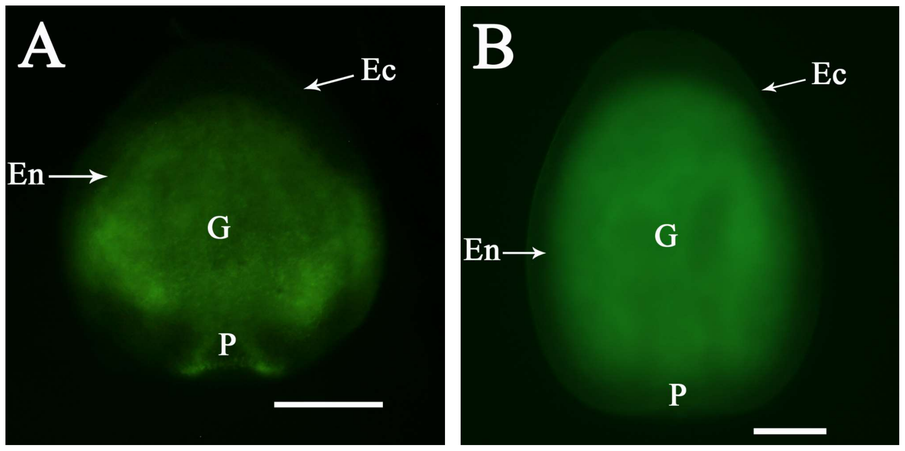

Fig 2A: Light micrograph of a planula exposed to FITC-BSA for 2 h. **2B**: Light micrograph of a planula exposed to FITC-Dextran for 5 h. G= gastrovascular cavity, P= pharynx, En= endoderm, Ec= ectoderm. Scale bars = 50 µm.

Discussion-

Earlier works with monomeric forms of DOM have shown ectodermal assimilation of amino acids and monosaccharides (e.g., Chia 1972, Schlichter 1982) by larvae of anthozoan cnidarians. Planula larvae of *N. vectensis* exposed to proteins ingested this macromolecular form of DOM into their GVC and these proteins were assimilated. The movement of seawater containing DOM into the GVC is presumably caused by the activity of pharyngeal cilia; dead larvae exposed to ferritin and iron dextran showed no label within the GVC, but the label was adsorbed to the outer epithelium (data not shown). The presence of discrete blue spots (reaction product) within cells of larvae exposed to ferritin suggests pinocytosis/endocytosis as the mode of assimilation. We interpret the diffuse fluorescence present within the endoderm of larvae exposed to FITC-BSA as evidence for the enzymatic separation of the fluorescein label from the protein – a process that could occur intra- and extracellularly.

The pattern of polysaccharide processing indicates that macromolecular assimilation by endodermal cells of planulae exhibits some degree of selectivity. Although larvae exposed to polysaccharides in seawater contained the label their within GVC, we were unable to detect assimilation of different macromolecular forms of DOM.

We were unable to detect the presence of particulate materials within the GVC of planulae of *N. vectensis*. The cause for this apparent discrepancy between fluid flow (DOM) and particle ingestion may relate to the selective exclusion of provided particles (e.g., Riggs 1988, Siebert 1974, Schwartz et al. 2002). Future experiments where particles and DOM are simultaneously presented to larvae are necessary to discriminate between these hypotheses.

Our results do not support particle capture as a significant feeding strategy for planulae of *N. vectensis*. Assimilation of dissolved macromolecules (proteins, but not polysaccharides) and monomeric forms of DOM (e.g., Chia 1972, Schlichter 1982) could be an avenue for assimilation of "food" from the environment. Feeding by anthozoan planulae on certain forms of DOM could affect larval or juvenile survivorship by decreasing the dependence on materials initially present within the egg.

Literature Cited-

Chia, F.-S. (1972). Note on the assimilation of glucose and glycine from seawater by the embryos of a sea anemone, *Actinia equine*. *Can. J. Zool.,* 50: 1333-1334.

Fautin, D.G, & Mariscal, R.N. (1991). Cnidaria: Anthozoa; in Harrison FW & Westfall, J (eds): Microscopic Anatomy of Invertebrates. Alan R. Liss, Chichester: pages 267-358

Riggs, L. (1989). Feeding behavior in *Aiptasia tagetes* (Duchassaing and Michelotti) planulae: A plausible mechanism for zooxanthellae infection of aposymbiotic planktotrophic planulae. *Journal of Veterinary Faculty University of Tehran.* 24(3-4), 201-206.

Schlichter D. (1975). The importance of dissolved organic compounds in sea water for the nutrition of *Anemonia sulcata* Pennant; in Barnes H (ed): Proceedings of 9th European Marine Biology Symposium. Aberdeen, Aberdeen University Press: pages 395-405.

Schlichter, D. (1982). Nutritional strategies of cnidarians - the absorption, translocation and utilization of dissolved nutrients by *Heteroxenia fuscescens*. *Am. Zool., 22*(3), 659-669.

Schwarz, J., Weis, V., & Potts, D. (2002). Feeding behavior and acquisition of zooxanthellae by planula larvae of the sea anemone *Anthopleura elegantissima*. *Mar. Biol.*, 140(3), 471-478.

Siebert, A.E., Jr. (1974). A description of the embryology, larval development, and feeding of the sea anemones *Anthopleura elegantissima* and *A. xanthogrammica. Can. J. Zool.*, 52(11), 1383-8.

Van Praët, M. (1990). Food intake and digestion in sea anemones; in Mellinger, J (ed): Animal nutrition and transport processes 1. Nutrition in Wild and Domestic Animals, Basel, Karger. Pages 24-35.

Weis, V. M.; Reynolds, W.S.; deBoer, M.D.; Krupp, D.A. (2001). Host-symbiont specificity during onset of symbiosis between the dinoflagellates *Symbiodinium* spp. and planula larvae of the scleractinian coral *Fungia scutaria*. *Coral Reefs.,* 20, 301-308.